Composition of Bovine γ-Caseins A¹ and A³, and Further Evidence For a Relationship in Biosynthesis of γ- and β-Caseins

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Abstract

Two variants, A¹ and A³, of γ- and β-caseins were isolated from samples of bovine milk which were typed as homozygous for β-casein A¹ or A³. γ- and β-Caseins A¹ and A³ differ in amino acid composition by two residues of histidine and the data suggest that the same substitutions, His/Gln and His/Gln or His/Pro distinguish the γ- and β-variant pairs. γ- β-casein polymorphs A¹, A², A³ and B all have a common C-terminal sequence -Ile-Ile-Val•OH and they show similar chymotryptic peptide maps. They differ in their N-terminal amino acids: arginine for β-caseins and lysine for γ-caseins. γ-Casein is smaller than β-casein by about 28 amino acid residues. It is possible that γ-casein is identical with a large portion of β-casein.

Introduction

In an earlier paper (3) we have summarized evidence which suggests strongly that bovine γ-casein is the same as a large fragment of bovine β-casein. Some of the data, which led to the formulation of this hypothesis, are reported here.

Previously published work (6) showed that the biosynthesis of γ-casein, like that of β-casein, is genetically controlled. Samples of milks from individual cows typed β-casein A¹, A², A³, and B always contained corresponding γ-casein polymorphs designated A¹, A², A³ and B, but no γ-casein was found in milks typed β-C. From the results of amino acid analysis of β- and γ-caseins A² and B, it was evident that the β- and γ-caseins were closely related chemically. The same differences in amino acid composition which distinguished the β-caseins, presumably two substitutions, Ser → Arg and Pro → His, also were found in the γ-polymorphs (5).

In the present paper we report new measurements of the molecular weights of β- and γ-caseins, which have necessitated a revision of previous ideas regarding their comparative size. We also describe amino acid analyses of β- and γ-caseins A¹ and A³, and chymotryptic peptide maps and end-group determinations of various β- and γ-polymorphs. The results of these experiments constitute additional evidence for compositional and structural similarity in these components of micellar casein.

Experimental Procedure

γ- and β-Caseins. Isolation of genetic variants of γ- and β-caseins, A¹, A², A³ and B from milks of individual cows has been described (5, 6). Proteins were isolated from caseins homozygous with respect to each protein. Purified proteins gave single bands by disc gel electrophoresis run in the presence of urea.

Molecular weight. Comparative measurements of molecular weights of β- and γ-caseins in dilute buffer solutions were made by sedimentation equilibrium as described previously (7). Guanidine•HCl solutions were used in other runs.

Molecular weights were also determined by gel electrophoresis in the presence of sodium dodecyl sulfate according to the method of Weber and Osborn (26).

Amino acid composition. Procedures of Moore and Stein (15) were used for automated amino acid analysis. In some experiments phenol was added to protein samples before acid hydrolysis to minimize destruction of tyrosine (24).

Tryptophan. Tryptophan analyses were made on γ- and β-caseins by Procedure U of Spies (25).

Phosphorus. γ-Caseins A¹ and A³ were analyzed for phosphorus according to Meun and Smith (14). Two independent determinations were made on each polymorph.

Peptide maps. γ- and β-Caseins were digested by chymotrypsin and their peptide patterns were compared by ninhydrin and specific staining technics as described by Kalan et al. (8).

Amino-terminal amino acids. N-Terminal residues of γ-, β-caseins A¹, A², A³ and B were

determined with dansyl chloride according to Gros and Labouesse (4). Dansylated protein was recovered by precipitation with trichloroacetic acid as described (4) or by filtration on a Sephadex G-25 column, the eluate being monitored for fluorescent protein derivative. Recovered dansylated protein was hydrolyzed in sealed evacuated tubes with 6 N HCl at 110 C for 4 hr.

High voltage electrophoresis separated and identified the dansyl derivatives. Neutral amino acid derivatives were extracted with ethyl acetate saturated with water because they were often obscured during electrophoresis by a strong band of dansyl sulfonic acid. Good resolution of the neutral amino acids was obtained at pH 4.38; arginine was resolved clearly at both pH 1.9 and 12.7. Didansyl lysine was resolved at pH 1.9 after electrophoresis of the ethyl acetate extract.

N-Terminal amino acid in γ-casein A^2 was also determined by the fluorodinitrobenzene method (2).

Carboxyl-terminal amino acids. C-Terminal amino acids in γ-, β-caseins A^1, A^2, A^3 and B were determined with carboxypeptidase A by the method of Kalan et al. (10), using a substrate to enzyme weight ratio of 75:1.

Results

Molecular weights and compositional analysis. In an earlier paper on the composition of γ- and β-caseins, Groves and Gordon (5) concluded, largely on the basis of minimal molecular weights calculated from tryptophan analyses, that γ-casein was the larger molecule with a molecular weight of about 25,000. Newer evidence, however, indicates that γ-casein is somewhat smaller than β-casein. Groves and Townend (7) reported molecular weights of about 22,000 for γ-casein A^3 and 24,700 (extrapolated to zero concentration) for γ-A^1 from measurements of sedimentation equilibrium in dilute aqueous solutions. More recent measurements in guanidine·HCl gave a similar value of 22,000 for γ-casein A^3. Furthermore, comparative runs with γ- and β-caseins under similar conditions in the ultracentrifuge indicate that γ-casein is the smaller molecule (Townend and Groves, unpublished results).

Because of the difficulties encountered by various investigators as well as by us in determining the exact molecular weights of caseins by sedimentation methods, estimates of comparative molecular weights of γ- and β-caseins A^2 from mobilities in dodecyl sulfate-gel electrophoresis were made. The results are in Figure 1. Also shown is a molecular weight of about 12,000 for minor components of casein, TS-, R- and S-caseins (5, 7). Although a molecular weight of 27,000 for β-casein is

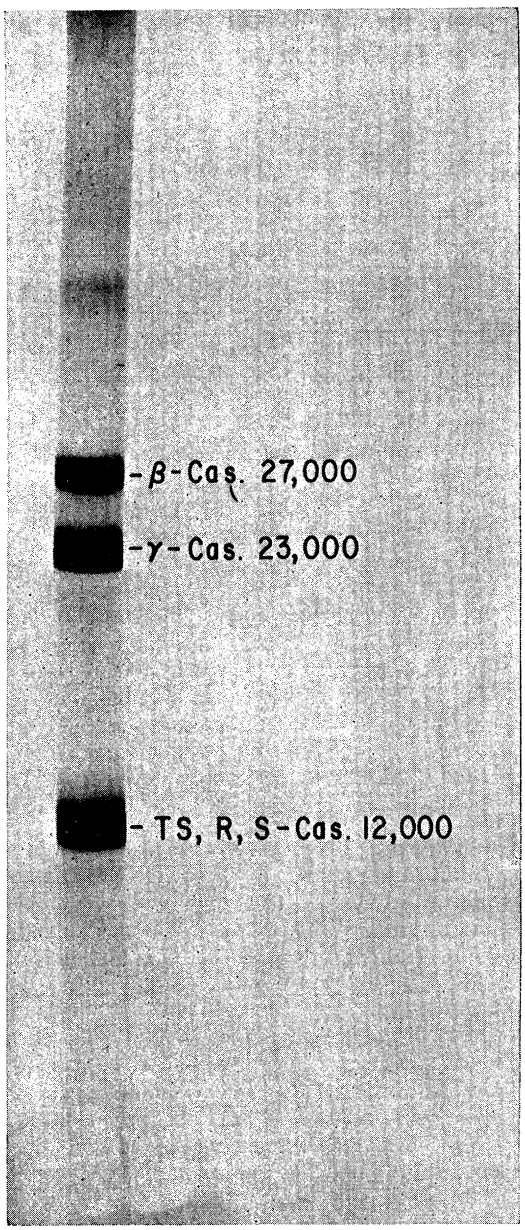

FIG. 1. Molecular weights of γ-, β-caseins together with TS-, R- and S-caseins by disc gel electrophoresis with sodium dodecyl sulfate. Anode is at the bottom.

higher than the generally accepted weight of 24 to 25,000 (16, 17, 22), the 23,000 for γ-casein agrees well with results from sedimentation equilibrium. Again, it seems clear that γ-casein is significantly smaller than β-casein.

As mentioned, previously published tryptophan analyses of γ- and β-caseins led us to believe that γ-casein was somewhat larger than β-casein. β-Caseins A and B had been reported to contain .876 and .832% tryptophan. γ-Caseins A^2 and B, prepared some years later from different samples of milk contained .82% tryptophan. We have now analyzed, simultaneously, preparations of β- and γ-casein polymorphs isolated from caseins homozygous with respect to each protein. Duplicate analyses of each sample gave averages for γ-caseins A^1, A^2, A^3 and B, of .81, .85, .90 and .84%, tryptophan, respectively, and for the corresponding β-casein polymorphs, of .78, .74, .75 and .74%. For each genetic type, γ-casein is a little richer in tryptophan than β-casein. We cannot explain why the figures for β-casein are now lower than before, but we are dealing with very small differences in concentration. If the present results are averaged and minimum molecular weights are then calculated, values of 24,000 for γ-casein and 27,100 for β-casein are obtained.

Our reported amino acid compositions (5) of γ-casein A^2 and B were derived from mean molar ratios and based on the presence of five glycine and six alanine residues per molecule. From these data a molecular weight of about 25,000 was calculated for the γ-caseins, somewhat greater than that for β-caseins, 23 to 24,000, similarly calculated but based on five glycine and five alanine residues per molecule. Since size of the β-casein molecule is well established (16, 17, 22) and substantiated by studies of its primary structure (23), and since γ-casein is apparently smaller than β-casein, we revise our calculations of numbers of amino acid residues per molecule of γ-casein, using as the base for molar ratio

TABLE 1. Amino acid composition of γ- and β-caseins[a].

	Residues amino acid per molecule calculated from mean molar ratio based on Gly = 4, Ala = 5				Gly = 5, Ala = 5			
Amino acid	γ-A^1	SD	γ-A^3	SD	β-A^1	SD	β-A^3	SD
Lys	9.6	0.13	9.7	0.09	10.8	0.12	10.8	0.39
His	5.6	0.09	3.9	0.06	5.7	0.09	3.8	0.16
Amide NH_3	25.0	0.50	24.7	0.69	26.8	0.72	27.2	0.42
Arg	1.8	0.00	1.9	0.03	3.8	0.04	3.8	0.13
Asp	7.0	0.08	7.1	0.08	9.1	0.11	9.0	0.13
Thr	7.9	0.08	8.0	0.10	9.0	0.13	8.9	0.08
Ser	10.6	0.11	10.7	0.14	15.1	0.19	14.9	0.11
Glu	31.5	0.41	33.0	0.35	39.0	0.70	39.5	0.50
Pro	32.6	0.83	34.0	0.55	34.2	0.85	35.1	1.32
Gly	4.1		4.1		5.0		5.0	
Ala	4.9		4.9		5.0		5.0	
Cys	0		0		0		0	
Val	16.3	0.23	16.5	0.17	18.6	0.43	18.8	1.02
Met	5.8	0.08	6.0	0.10	5.9	0.21	5.8	0.20
Ile	6.6	0.06	6.6	0.13	9.7	0.14	9.7	0.31
Leu	18.6	0.21	19.1	0.22	21.7	0.23	21.7	0.43
Tyr	3.1	0.22	3.5	0.12	3.6	0.19	3.6	0.26
Phe	8.7	0.10	8.8	0.12	8.8	0.10	8.8	0.21
Trp	0.8		0.9		0.9		0.9	

[a] Residue numbers listed are averages of, or extrapolated values from, nine determinations. Triplicate analyses were made on samples hydrolyzed 24, 72 and 96 hr. For valine and isoleucine the figures are averages of the six determinations made on the longer-term hydrolyzates. Threonine, serine and amide ammonia numbers were obtained by linear regression analysis, and for these the standard error is shown rather than the standard deviation. Figures for tryptophan are derived from analyses described in the text and from calculated molecular weights of 20,000 for γ- and 23,500 for β-casein. Amino acid residue numbers reflecting the possible substitutions which differentiate A^1 and A^3 variants are underlined.

TABLE 2. Comparison of composition of γ- and β-casein.

Amino acid	Whole number residues per molecule containing 5 Ala								
	γ-A^1	γ-A^{2a}	γ-A^3	γ-B^a	β-A^{1b}	β-A^{2b}	β-A^3	β-B^b	β-C^b
Lys	10	10	10	10	11	11	11	11	12
His	6	5	4	6	6	5	4	6	6
Arg	2	2	2	3	4	4	4	5	4
Asp	7	7	7	7	9	9	9	9	9
Thr	8	8	8	8	9	9	9	9	9
Ser	10-11	10-11	10-11	9-10	15	15	15	14	15
Glu	31-32	32	33	32	38-39	39	39-40	39	37
Pro	32-33	33	34	32	33-35	34	34-36	33	33
Gly	4	4	4	4	5	5	5	5	5
Ala	5	5	5	5	5	5	5	5	5
Val	16-17	16-17	16-17	16-17	18-19	18-19	18-19	18-19	18-19
Met	6	6	6	6	6	6	6	6	6
Ile	7	7	7	7	10	10	10	10	10
Leu	19	19	19	19	21-22	21-22	21-22	21-22	21-22
Tyr[c]	4	4	4	4	4	4	4	4	4
Phe	9	9	9	9	9	9	9	9	9
Trp	1	1	1	1	1	1	1	1	1
P	1	1	1	1	5	5	5	5	4

[a] Numbers recalculated from previous data (5).
[b] Previously published data (5), revised slightly to reflect results of additional analyses.
[c] See text.

computation the presence of four glycines and five alanines per molecule. The results of amino acid analysis listed in Table 1 and the rounded residue numbers used for the comparisons in Table 2 were obtained in this way. The molecular weight of γ-casein calculated from these data is about 20,000.

Phosphorus of both γ-caseins A^1 and A^3 was .15%. From this a minimum molecular weight of about 21,000 may be calculated. We have previously reported (5) analyses of .17 and .16% P for variants A^2 and B.

Peptide patterns and specific staining. The electrophoretic patterns before ascending chromatography, together with the peptide maps of γ-, β-caseins A^2, B and A^1, A^3 are in Figures 2 and 3. The patterns for the γ- and β-caseins are similar and at least 19 corresponding peptides, as determined by ninhydrin, are found in all the polymorphs. There are four peptide differences between the γ- and β-caseins: peptides 5 and 33 are present in all γ-caseins but absent in β-caseins, and peptides 6 and 39 are absent in the γ-caseins but present in the β-caseins. Peptide 6, found only in the β-caseins, is strongly acidic based on its electrophoretic mobility. Since β-casein contains more phosphorus than γ-casein, Peptide 6 is probably the phosphopeptide.

A number of peptides are not found in all types of γ- and β-casein. For γ-, β-caseins typed B, Peptides 1, 2, 3, 4, 11, 40 and 41 are absent; For A^1, Peptides 2, 25, 40, 41 and 42 are absent; for A^2, Peptides 36, 40 and 41 are absent; for A^3, Peptides 3, 4, 25, 30, 36 and 42 are absent. Also, only the A^2 types of γ-, β-caseins contain Peptides 7, 8 and 9. There are a number of peptides, 4, 10, 11, 13, 15, 16 and 17, which are absent in only one or two types of either the γ- or β-caseins.

By specific staining reactions it was shown that Peptide 19 is the only one staining for tryptophan and it is found in all the caseins. This is consistent with the finding that the γ- and β-caseins all contain one tryptophan per molecule.

Specific staining of the peptides also demonstrates the similarities between γ- and β-caseins. For all the polymorphs, Peptides 10, 12, 24, 26 and 31 contain methionine, Peptides 23 and 35 contain tyrosine, Peptides 22 and 24 contain arginine and Peptides 26, 27 and 31 contain histidine.

Peptide 6 contains histidine and arginine and, as already mentioned, this peptide is not found in the γ-caseins. Also, Peptide 33, absent in the β-caseins, contains histidine in all the γ-caseins except A^1.

Peptides 13 and 15 appear to be difference peptides. Peptide 15 is found only in γ-casein A^2 whereas Peptide 13 is present in all the polymorphs except γ-A^2. Furthermore, Peptides 13 and 15 both contain histidine and methionine. Peptide 41 is found only in the

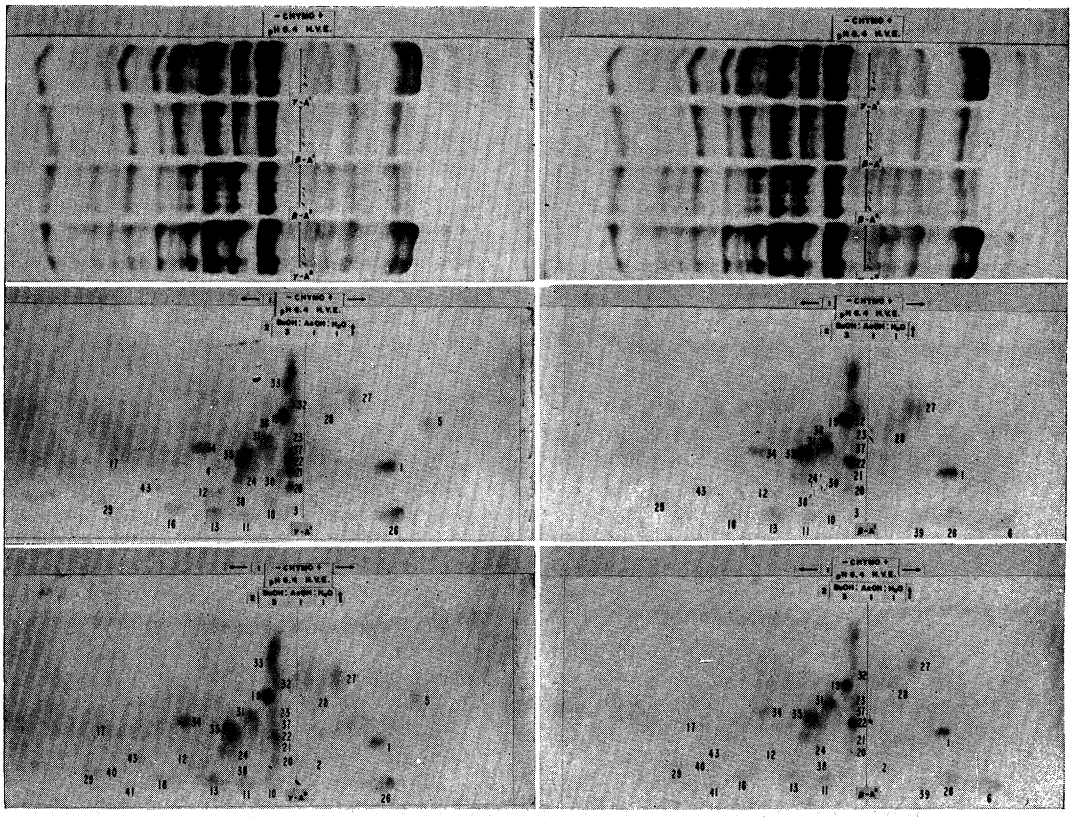

FIG. 2. Peptide patterns from chymotryptic digests of γ-, β-caseins A^2 and B. Top photograph shows one-dimensional high-voltage paper electrophoresis at pH 6.4, 40 v/cm 2 hr. Other four photographs are maps produced by two-dimensional electrophoresis and chromatography. Peptides located in equivalent positions are given the same number.

γ-, β-casein A^3 polymorphs whereas Peptide 30 is found in all except the A^3 types. Since both Peptides 30 and 41 contain methionine, they also might be difference peptides.

Amino- and carboxyl-terminal amino acid determinations. Arginine was N-terminal in all the β-casein polymorphs examined by the dansylation method. This finding is in agreement with earlier results on β-caseins A, B and C (10, 11).

Arginine was previously reported, erroneously, to be the N-terminal amino acid of all γ-casein polymorphs (9). Re-examination of the N-terminal amino acid by a variation of the dansylation technic of Gros and Labouesse (4) has shown that lysine is the N-terminal amino acid for the four γ-caseins. The variation entailed extensive extraction of the dansylated protein hydrolysate with water-saturated ethyl acetate and examination of the extract by high voltage electrophoresis at pH 1.9. Under these conditions, didansyl lysine was resolved from dansyl sulfonic acid and was shown to have a mobility corresponding exactly to that of a known sample of didansyl lysine. This is in agreement with our finding of lysine as N-terminal amino acid of γ-casein A^2 by the DNP-method, and the finding of N-terminal lysine for γ-casein A^3 on the amino acid sequencer (3).

The same C-terminal sequence, Ile-Ile-Val•OH was found in γ-, β-caseins A^1, A^2, A^3 and B by hydrolysis with carboxypeptidase A. Table 3 shows a typical time study for γ-, β-caseins A^3. The other polymorphs show a similar release of valine and isoleucine. Release of valine predominates at very short times. Isoleucine is released at a slightly later time and becomes predominant in total quantity of amino acids recovered. The γ-caseins released an amount of isoleucine in excess of 2 moles/mole protein. The β-caseins released a smaller quantity of isoleucine, about 1.6 moles/mole protein. The release of both valine

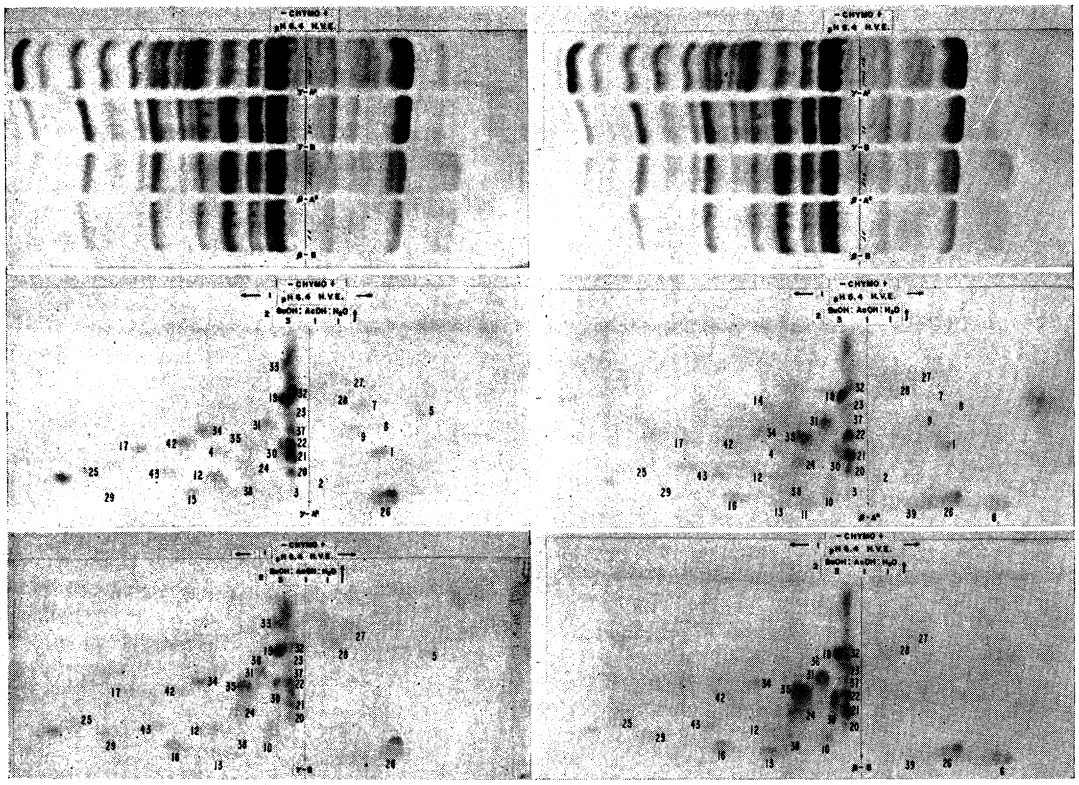

FIG. 3. Peptide patterns from chymotryptic digests of γ-, β-caseins A^1 and A^3. Conditions of electrophoresis, chromatography, and peptide numbering are the same as in Figure 2.

and isoleucine in γ-caseins was significantly greater than in β-caseins. The reason for release of isoleucine in excess of 2 moles/mole of protein is unknown. Other amino acids were liberated in small amounts, namely serine, glycine, alanine, and leucine.

Discussion

Estimates of the molecular weights of γ- and β-caseins by different methods have not yielded entirely consistent results. For γ-casein the molecular weights have ranged from 21,000 to 25,000 and for β-casein, from 24,000 to 27,000. However, comparative measurements under the same conditions have indicated uniformly that β-casein is the larger molecule. Therefore, as explained before, we have calculated the results of amino acid analysis on the basis in Table 1 and have revised our previous analyses (5) of γ-casein A^2 and B accordingly (Table 2).

It may be seen from the results in Table 1 that the only certain difference in amino acid composition between γ-caseins A^1 and A^3 is in

TABLE 3. Amino acids released from γ-, β-caseins A^3 by carboxypeptidase A.

Moles amino acids released/20,000 g γ-casein A^3	Time, hr				
	0.25	1.0	4.0	8.0	24.0
Val	0.11	1.25	1.41	1.48	1.40
Ile	0.09	1.48	2.63	2.86	2.80
Moles amino acids released/24,000 g β-casein A^3					
Val	0.47	0.93	0.97	1.00	0.90
Ile	0.43	1.21	1.51	1.60	1.49

content of histidine, close to two residues per molecule; and the same difference distinguishes β-caseins A^1 and A^3. Presumably, we are dealing with genetically controlled amino acid substitutions but these cannot be specified surely from present data. A His→Gln substitution seems very probable for the γ-casein pair and possible for the β-casein polymorphs. A second substitution, His→Pro, is also suggested by the results for both γ- and β-caseins. There are no other significant differences in composition between γ-caseins A^1 and A^3 nor between β-caseins A^1 and A^3.

For further comparisons the figures in Table 1 have been rounded to the whole numbers shown in Table 2. The listing of four tyrosine residues per molecule of each variant of γ- and β-casein should be explained. Some destruction of tyrosine during acid hydrolysis of the caseins was not unexpected, but deviation of analytical results from whole number values, as in the analyses in Table 1, was serious. Accordingly, additional determinations of tyrosine were made by two methods: spectrophotometrically in solutions of the proteins, and in the amino acid analyzer following acid hydrolysis in the presence of phenol. No differences in tyrosine content among the γ- or β-casein variants could be detected spectrophotometrically. Appreciably less destruction of tyrosine occurred during acid hydrolysis when phenol was present: for γ-casein A^1 and A^3, 3.8 and 3.9 residues per molecule were found; for β-caseins A^1, A^3 and two samples of β-C, 3.9, 3.9, 3.8 and 4.0 residues per molecule were found. One of the samples of β-casein C was obtained from milk typed β-A^2C; the other, previously analyzed and found to contain three residues per molecule, was prepared from milk typed β-CC (5). It appears that, with destruction of tyrosine minimized, all variants of β- and γ-caseins isolated in the present investigation contain four residues tyrosine per molecule.

Incidentally, finding of a smaller molecular weight for γ-casein has improved the stoichiometry of the arginine analyses. Previous results (5) of 2.4 and 3.6 residues per molecule for γ-caseins A^2 and B, arbitrarily rounded to 3 and 4, can now be revised to 2.0 and 2.9 before rounding.

To allow comparisons of pairs of variants of γ- and β-caseins, all of the data in the present study are listed together in Table 2. In some instances, it has been impossible for obvious reasons to assign a whole number value with confidence. Nevertheless, the data for the β-caseins are in essential agreement with earlier analyses by Peterson et al. (11, 18) and suggest the same amino acid substitutions previously postulated for these variants. Comparisons of polymorph pairs are made in Table 4, but those involving β-casein C are not shown because a corresponding γ-casein variant has never been detected. All substitutions inferred from the data for β-caseins are also suggested by the results for the γ-caseins. Thus, the close relationship in biosynthesis of γ- and β-caseins is again evident.

TABLE 4. Possible amino acid substitutions common to γ- and β-caseins.

Comparison	Substitution
A^1-A^2	His/Pro
A^1-A^3	His/Gln, His/Gln or His/Pro
A^1-B	Ser/Arg
A^2-A^3	His/Gln or His/Pro[a]
A^2-B	Pro/His, Ser/Arg
A^3-B	Ser/Arg, Pro/His, Gln/His or Pro/His

[a] Substitution in these β-casein variants is His/Gln according to Ribadeau Dumas et al. (21).

The differences in amino acid composition between γ- and β-caseins noted in our earlier report (5) must now be redefined from the data in Tables 1 and 2. Per molecule, β-casein is larger by 1 Lys, 2 Arg, 2 Asp, 1 Thr, 4 to 5 Ser, 7 Glu, 2 Val, 3 Ile, 2 to 3 Leu and probably 1 Pro residues; also by four atoms of P. If these 26 to 28 residues and four P atoms were linked together in the form of a phosphopeptide, the peptide would be very similar in composition to the 24-residue tryptic peptide prepared from β-casein by Peterson et al. (19) in 1958, and thought to be N-terminal in the β-casein molecule. More recently, the same phosphopeptide was prepared from β-casein A^2 by Ribadeau Dumas et al. (20) and from β-casein A^1 by Manson and Annan (13). In each case its amino acid composition was Arg_2 Asp_1 Thr_1 $SerP_4$ Ser_1 Glu_7 Pro_1 Gly_1 Val_2 Ile_2 Leu_3, 25 residues in all. The same sequence of amino acids was established by each group of investigators and each positioned the phosphopeptide as N-terminal in β-casein, residues 1 to 25. Returning now to the present data, if we accept the higher total of 28 amino acid residues as the difference between β-casein and γ-casein, β-casein may be thought of as γ-casein + phosphopeptide + a tripeptide, -Ile-Asp-Lys-. This tripeptide has, in fact, been isolated from β-casein A^2 as tryptic peptide, T9, by Ribadeau Dumas et al. (22), sequenced, and ordered as residues 26 to 28 in the N-terminal portion of β-casein

(23). Could γ-casein be the remaining part of β-casein, residues 29 to 209? If so, the N-terminal group of γ-casein would be Lys, residue 29 in the β-casein sequence (20). Our results show that lysine is, indeed, N-terminal in all γ-casein polymorphs. Also, if so, the C-terminal group would be the same. We have found that the identical C-terminal sequence, -Ile-Ile-Val•OH, is present in all the β- and γ-casein variants examined. Furthermore, the many similarities found in peptide mapping not only point up the close chemical relationship between β- and γ-caseins, but would, of course, be in harmony with the idea that portions of the molecules are identical. Additional sequencing of the N-terminal end of γ-casein A^3, residues 1 to 16, has given results (3) completely compatible [1] with the sequence of residues 29 to 47 in β-casein A^2 partially worked out by Ribadeau Dumas et al. (1, 20, 23). More sequencing will be needed to verify the idea that γ-casein is a large fragment of the β-casein molecule.

The present report raises at least two questions. First, if it is true that γ-casein has the same primary sequence as β-casein except for the absence of the N-terminal phosphopeptide and a tripeptide, then one can ask how γ-casein is related to β-casein in a biosynthetic sense. Second, if it is true that all γ- and β-casein polymorphs are related biosynthetically in the same mechanistic fashion, then how can one explain the absence of a γ-casein C variant.

In response to the aforementioned questions, at least two hypotheses can be proposed. Each of the γ-casein proteins may arise as the result of a highly specific limited proteolysis of corresponding β-casein variants. This would suggest that the protease responsible is inhibited in its action on β-casein C so that the corresponding γ-casein is never formed. This concept would explain the formation or lack of formation of γ-casein variants at a level removed from the protein synthesizing apparatus.

An alternate explanation would involve the protein synthesizing apparatus and suggest a single gene locus responsible for the production of both β- and γ-casein polymorphs. In this case, the production of the messenger RNA coding for the γ-caseins may be controlled by some unknown transcription mechanism or a single messenger RNA for β-caseins may be translated in some unknown way to give rise to γ-casein variants in this process. Absence of the γ-casein C polymorph may then be attributed to some deficiency in the transcription or translation of the corresponding β-casein C messenger RNA.

These hypotheses by no means exhaust all possibilities of explaining the relationship of the β- and γ-caseins. Further experimental data are required to disclose the actual mechanism by which these two groups of proteins are seemingly related. At present, however, it seems impossible to reconcile our findings with the lack of synchrony in the biosynthesis of β- and γ-caseins suggested by the data of Larson and Gillespie (12), even though γ-casein, as defined in 1957, was different from the γ-casein currently being investigated.

Acknowledgments

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[1] In our previous paper (3) we identified, provisionally, the seventh amino acid residue in γ-casein A^3 as -Ser?-. Subsequently, this residue has been identified unequivocally by Peterson, Groves, and Mitchell as phosphoserine not only in γ-casein A^3 but also in γ-casein A^2; experimental details will be published elsewhere. Thus, Residue 7 in γ-casein is the same as Residue 35 in β-casein A^2 (1).

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